BURDEN OF HOSPITAL ACQUIRED INFECTIONS AND ANTIMICROBIAL USE IN LIBYA HOSPITALS ADULT INTENSIVE CARE UNITS

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ABSTRACT

Evaluate drug resistance of bacteria isolated from equipment placed close to patients in an Intensive Care Unit of a Central hospital in Gharian.Libya is a lower middle-income country with no national surveillance system for hospital-acquired infections (HAIs). We assessed the prevalence of hospital-acquired infections and antimicrobial use in adult intensive care units (ICUs) in Libya.This is a cross-sectional study. The samples were collected with swabs moistened with Trypticase Soy Broth, which were then cultured in sheep blood agar and MacConkey agar. The phenotypic identification performed was based on the morphology of the strains and biochemical results. The drugs resistance analysis was based on Kirby-Bauer's Disk Diffusion protocol. Results:A rate of 94.4% of the analyzed equipment was contaminated. The most frequently isolated microorganisms were: *Acinetobacter* sp., *Staphylococcus aureus* and *Pseudomonas* sp. Just about 75% of *Acinetobacter* sp. was resistant to piperacillin associated to tazobactam, meropenem and levofloxacin. Similarly, 36.3% of *S. aureus* showed resistance to oxacillin and 10% of *Pseudomonas* sp. was resistant to the drugs tested.

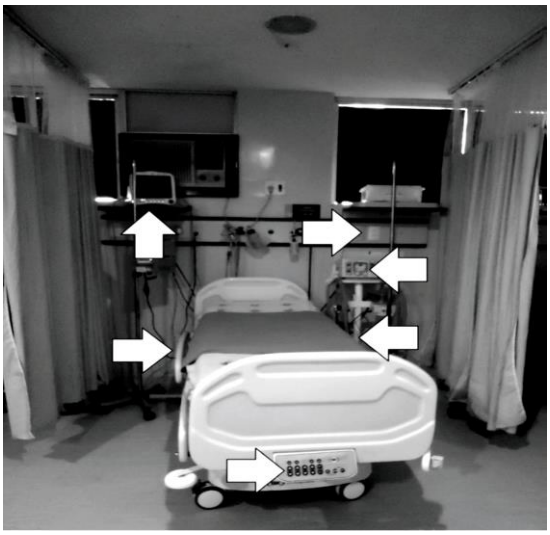
INTRODUCTION

Healthcare-associated Infections (HAI) are responsible for thousands of deaths every year around the world. In Libya, this problem increases in terms of numbers as well as complexity, causing economic and social disruption with high levels of morbidity and mortality[1,2]. In Intensive Care Units (ICU), the contamination of equipment by bacteria is common, turning them into reservoirs of these microorganisms,enabling the colonization and cross infection of patients, complicating prognosis and favoring HAI outbreaks, mostly by microorganisms multiresistant to antibiotics commonly applied in therapeutics[3], which implies severe limitations to the treatment of hospital infections, posing a great threat to public health[4].

Different organisms are related to contaminations in hospital environments and HAI processes[5], but the main pathogens include oxacillin-resistant *Staphylococcus aureus* (ORSA), vancomycin-resistant *Enterococcus* sp. (VRE) and, more recently, extended-spectrum beta-lactamases (ESBL) andcarbapenem-resistant *Acinetobacter baumannii*[6-9]. Bacterial resistance is natural and unavoidable[3], but the frequent and undistinguished use of antimicrobials (mainly broad-spectrum drugs) are crucial factors for the development and acceleration of this process[3,10]. Given these facts, the purpose of this study was to isolate and determine the drug resistance profile of bacteria isolated from ICU equipment in a hospital in Gharian.

MATERIAL AND METHODS

This descriptive and cross-sectional study was developed in the Intensive Care Unit of a Central hospital located in Gharian, in the Aljabal region of Libya, from January to December 2014. Convenience sampling was applied, in which 54 pieces of equipment (right and left side rails and height adjustment buttons from the beds, infusion pump buttons, individual light switches and cardiac monitor shelves) distributed among the nine beds present in the general ICU (Figure 1) were selected for collection. The inclusion criterion was samples from surfaces whose beds were occupied by their respective patients.



(Figure 1) Equipment collected (arrows) in each bed

The collected data were typed, validated and processed in the software Excel 2010 (Microsoft Office). Descriptive analysis was applied to obtain the percentage of samples.

The samples were collected six hours after the last time the hospital beds had been cleaned (corresponding to two hours after the end of the visiting period), so as not to interfere in the routine activities on site. Sterile swabs were used moistened with Trypticase Soy Broth (TSB) medium. Immediately after the collection, when the swabs were spun around their axis over the previously selected equipment, they were

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again stored in the medium and incubated at 36 ± 0.5oC during 24 hours.

After the growth in TSB, the samples were seeded in sheep blood agar and MacConkey agar and also incubated at 36±=0.5oC during 24 hours. Gram staining was performed, followed by the identification of the genera and/or species of the bacteria, according to macro and microscopic characteristics of the colonies and biochemical test results. For the identification of bacteria from the family *Enterobacteriaceae*, the carbohydrate fermentation test was used in Triple Sugar Iron (TSI), as well as biochemical tests using the Sulfide Indole Motility (SIM), Simmons' citrate and Christensen's Urea Agar growth mediums. Tests based on Oxidase and Polymyxin B were used for the identification of glucose-non-fermenting Gram-negative bacteria. The identification of *Staphylococcus* sp. was performed through catalase, DNase and Novobiocin tests. *Streptococcus* sp. were identified through the characteristics of hemolytic activity, the use of Bile esculin agar, Brain Heart infusion (BHI) + NaCl 6.5% and optochin tests.

The drugs resistance analysis was based on Kirby-Bauer's Disk Diffusion protocol in Mueller-Hinton agar, as proposed by the Clinical and Laboratory Standard Institute[11].

RESULTS

It was observed that 94.4% of the analyzed equipment was contaminated by one or more bacterial species. The most numerous isolated bacteria were *Acinetobacter* sp., *Staphylococcus aureus*, Coagulase- negative *Staphylococci* (CoNS), *Staphylococcus saprophyticus*, *Enterococcus* sp., *Klebsiella pneumonia* and *Streptococcus viridans*, as presented in (table 1). Gram-positive *bacilli* were found in 33.3% of the beds.

(Table 1) Distribution of bacteria isolated on equipment

Microorganism	S1 n (%)	S2 n (%)	S3 a (%)	S4 n (%)	S5 n (%)	S6 n (%)	Total n (%)
Acinetobacter sp.	03(18.7)	04(25)	03(18.7)	01(6.25)	01(625)	04(25)	**16(26.57)**
S. aureus	04(363)	02(18.1)	01(9)	01(8)	02(18.1)	01(9)	**11(19.64)**
Pseudomonas sp	01(10)	01(10)	02(20)	01(701)	01(10)	04(40)	**10(17.85)**
S coagulase negative	0(0)	0(0)	01 (14.2)	03(42.8)	02(28.5)	01(14.2)	**07(12.5)**
S. saprophyticus	0(0)	02(28.5)	03(42.8)	0(0)	01(14.2)	01(14.2)	**07(12.5)**
Enterococcus sp	0(0)	0(3)	0(0)	01(33.31)	01(33.3)	01(33.3)	**33(5.35)**
klebsiella pneumonia	0(0)	0(0)	0(0)	0(0)	0(0)	01(100)	**01(1.76)**
S. viridians	0(0)	0(0)	0(0)	0(0)	0(0)	01(100)	**01(1.78)**
Total Isolated	**08(14.2)**	**08(16)**	**10(17.8)**	**07(12.5)**	**08(142)**	**14(25)**	**56(100)**

S1-right rail; S2-left rail; S3 - height adjustment buttons from the beds; S4 - Infusion pomp buttons; S5 - individual illumination switches; S6 - cardiac monitors' shelf; N - number of isolated bacteria; %-percentage of isolated bacteria.

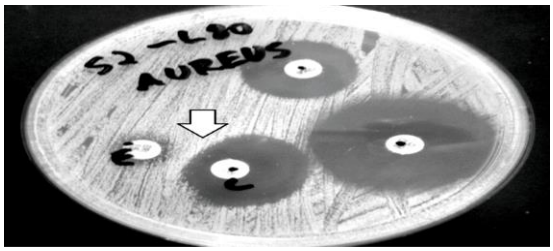
Among the *Acinetobacter sp.* isolated, 75% were resistant to imipenem, levofloxacin and piperacillin associated with tazobactam. 37.5% of the isolated microorganisms from this genus were resistant to ticarcillin, 31.2% to amikacin, 18.7% to ciprofloxacin, tetracycline and ceftazidime and 12.5% to gentamicin. 6.25% of the isolated microorganisms were found to have an intermediate level of resistance to ceftazidime and 12.5% to tobramycin.

With regard to the isolated *Staphylococcus aureus*, 72.7% were resistant to erythromycin, 63.6% to penicillin, 54.5% to clindamycin and ciprofloxacin and 18.8% to gentamicin. None of the isolated *Staphylococcus aureus* turned out to be resistant to cefoxitin. 9% of them had an intermediate level of resistance to oxacillin, clindamycin, erythromycin and ciprofloxacin. 36.3% were resistant to oxacillin (ORSA).

Among the Coagulase-negative *Staphylococci* strains, 71.4% and 54.1% were resistant to erythromycin and clindamycin, respectively. All these strains were susceptible to gentamicin, with 14.2% of them having the intermediate resistance phenotype to clindamycin. 42.8% were resistant to penicillin and 14.2% to tetracycline and cefoxitin.

The biggest number of cases of resistance to oxacillin is this study occurred in the isolated strains of *Staphylococcus saprophyticus*, which reached resistance levels in 85.7% of the samples, followed by 71.4% of resistance to erythromycin and clindamycin, 42.8% resistant to ciprofloxacin, 42.6% to cefoxitin, 28.5% to tetracycline and 14.2% to penicillin.

As regards the mechanism of inducible resistance to clindamycin, 12% of the *Staphylococcus* sp. had the positive phenotype. This was detected through the disk approximation test with erythromycin and clindamycin (Figure 2), in which 66.6% corresponded to Coagulase-negative *S.* and 33.3% to *S. aureus*.



(Figure 2) Inducible clindamycin resistance phenotype in *S. aureus*. E - erythromycin; C – clindamycin

Only 5.35% of the isolated strains corresponded to *Enterococcus* sp., 33.3% of which had intermediate levels of resistance to penicillin and ampicillin. None of the isolated strains were resistant to vancomycin.

A rate of 17.85% of the isolated strains corresponded to *Pseudomonas* sp., none of which turned out to have a significant level of resistance to the tested antibiotics (gentamicin, levofloxacin, aztreonam, ceftazidime, tobramycin, amikacin, ciprofloxacin, meropenem, chloramphenicol, cefoxitin and ticarcillin + clavulanic acid), while 10% of them were resistant and 20% presented intermediate levels of resistance to piperacillin + tazobactam; and 10% also had intermediate levels of resistance to ticarcillin + clavulanic acid and aztreonam.

Only one specimen of bacteria from the *Enterobacteriaceae* family (*Klebsiella pneumoniae*) was isolated in the study performed. Resistance was detected to ciprofloxacin, tetracycline, ampicillin, chloramphenicol and gentamicin, intermediate resistance to piperacillin + tazobactam and susceptibility to meropenem and tobramycin, and a negative result was obtained for Extended-Spectrum Beta-Lactamase (ESBL) production.

DISCUSSION

Part of the results reported in this study (be it the occurrence of genera and/or species of bacteria or their antimicrobial resistance profiles) corroborate what is described in the scientific literature, although comparisons sometimes tend to be inaccurate, since the sampling and microbial detection methods vary considerably among different studies[2].

The high number of *Acinetobacter* sp. Isolated may have been due to its high level of nutritional and metabolic versatility, which allows this genus to use a large variety of substrates as carbon sources, remaining active for days or weeks in hospital environments[12,13], which highlights the importance of their detection in such places, as this microorganism is directly involved in various ICU-related clinical complications[2], and also in mechanisms of acquired resistance to carbapenem antibiotics[4]. Despite the existence of studies[2,4,6,13] on the genus *Acinetobacter* as an HAI agent from clinical samples, there is a lack of statistical data on the prevalence of this bacterium on hospital equipment. Besides that, the occurrence[4,13,14] of *Acinetobacter* sp. with multiresistant profiles is also considerable, which shows the need for more complex studies to determine the behavior of these strains in this kind of environment.

As regards the resistance profile of *S. aureus* strains to oxacillin (ORSA), a low number of cases was detected compared to the number of Gram-positives, although the result turned out to be superior when compared to a similar study, in which only 11.8% of the isolated strains had this resistance profile[15]. When the resistance of *S. aureus* strains to cefoxitin is analyzed, the results described in this study were similar to those of a study performed with biological materials, in which no strains with this resistance profile were reported[16].

As for the Coagulase-negative *Staphylococci* strains, no studies on their resistance profile in ICU equipment were found, but a study from 2006[17] reported lower resistance levels to erythromycin and clindamycin in biological samples: 68.7% and 63% respectively.

The occurrence of *S. saprophyticus* described in this study was superior when compared to studies from Libya, performed in 2014, in which only 3.3% of the analyzed pieces of hospital equipment were contaminated with this bacterial species[18]. Although the authors did not publish data on the antimicrobial resistance profile of *S. saprophyticus*, the levels for the genus *Staphylococcus* corresponded to 71.4% and 38.1% for erythromycin and ciprofloxacin, respectively[18], corroborating the importance of their detection.

Despite the small number of *Staphylococcus* sp. strains with positive phenotype to induced resistance to clindamycin, this finding ought to be considered with special attention since the environment in question is one in which the patients are immunocompromised. Thus, the small number of occurrences must not be underestimated, as these bacteria have a great potential to cause hospital infections[2,3,5]. The low occurrence of vancomycin-resistant *Enterococcus* sp. strains differs from a study performed in the US using biological samples[19], in which the estimated rate of resistance to this drug in ICU was of 17.7%. In Brazil, the first report of this phenotype dates back to 1998 and, in Latin America, the rise in the number of cases of this kind of resistance happened in countries like Chile, Uruguay and Argentina[20,21].

The presence of *Pseudomonas* sp. strains as reported in this study corroborates what is reported in similar studies[22-24], based on the analysis of equipment from different hospital environments, including ICU. In a hospital environment, the biggest sources of contamination from this microorganism are breathing equipment, hemodialysis systems, sinks and cleaning apparatus. The relevance of *Pseudomonas* sp. As a potential hospital pathogen depends on the bacterial species and is associated to its relative resistance to the drugs, as well as to its reduced susceptibility to the antiseptics and disinfectants used in these environments[25,26]. The drugs resistance profile of *Pseudomonas* sp. here described corroborates previous studies performed with biological material[27], in which the major part of the antibiotics tested on *Pseudomonas aeruginosa* turned out to be effective against most of the isolated strains. The presence of these strains with mutually similar drug resistance profiles suggests the dissemination of a clone in the hospital environment; a fact that is probably related to cross-contamination mechanisms, although more extensive studies would be needed to confirm its dispersion in the ICU environment.

With regard to the percentage of isolated strains belonging to the *Enterobacteriaceae* family, the results obtained in this study differ from what is reported in studies performed in equipment from hospital environments[28], where 30.3% were found to be contaminated by strains of this family. On the other hand, the antimicrobial resistance analysis of these strains in this study corroborates what is reported in the scientific literature: the massive presence of resistance to aminoglycosides and third-generation cephalosporins[7,29].

When ESBL-production is considered, the results described in this study differ from those reported by Weber *et al*[30]. and Judge *et al.*[28], in which a total of 22.2% of *Enterobacteriaceae* strains isolated on equipment from ICUs beds were ESBL- producing.

CONCLUSIONS

High prevalence of HAIs in Libyan ICUs, mainly caused by Gram-negative bacteria with high levels of antimicrobial use illustrate the urgent need for capacity strengthening in both rational antimicrobial use and infection control efforts at national, regional and local levels. Most of the isolated strains had high rates of antimicrobial resistance to the drugs, which posses a great threat to public health

SUPPORTING INFORMATION

Hospital and ICU Characteristics, Patient Characteristics, HAI Prevalence per Month, Antimicrobials Combinations Used. The Common Antimicrobial Agents Used.

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